

D2

86. (Twice Amended) A process for stably transforming a target higher plant species which comprises introducing an integration and expression universal vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent to stably transform the chloroplast of higher plants and comprising an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest, and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence in the chloroplast genome of the target higher plant, a heterologous nucleotide sequence coding for a selectable phenotype, and flanking each side of the expression cassette, chloroplast DNA sequences of a higher plant which comprise each one a portion of the intergenic spacer 2 region between the tRNA^{Ile} and the tRNA^{Ala} genes of the chloroplast genome, said chloroplast sequences conserved in all higher plants and competent of undergoing homologous recombination with complementary spacer 2 sequences of heterologous target plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the complementary spacer 2 sequences of the target plant chloroplast genome.

D3

171. (Once Amended) The universal integration and expression vector of claim 190 which does not include a transposon.

190. (Once Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the

[Handwritten signature]

chloroplast genome of a target higher plant, and flanking each side of the expression cassette, chloroplast DNA sequences which originate from a plant species different from the target plant, said chloroplast sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which chloroplast sequences are also competent of undergoing homologous recombination with said complementary sequences, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated by said homologous recombination of the flanking sequences with the complementary sequences in the target chloroplast genome, and wherein said stable integration is not directed into a transcriptionally inactive region of the chloroplast genome.

191.(Once Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into a transcriptionally active region of the chloroplast genome of

the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome.

192.(Once Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences including a transcription termination region to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences

193. (Once Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking chloroplast DNA sequences each one a portion of a synthetic spacer 2 region between

the tRNA^{Ile} and tRNA^{Ala}, said chloroplast sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which chloroplast sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome.

194. (Once Amended) The process of claim 86 wherein the flanking sequences originate from other than the target plant and comprise, each one a portion of the intergenic spacer 2 region between the tRNA^{Ile} and the tRNA^{Ala} genes of the chloroplast genome, whereby double homologous recombination with the conserved spacer 2 region in the target chloroplast genome is facilitated.

196. (Once Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary

to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome, and wherein said stable integration is not directed into a transcriptionally inactive region of the chloroplast genome.

197. (Once Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into a transcriptionally active region of the

chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome.

8
D5
198. (Once Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence including a transcription termination region in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant and which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome.

199. (Once Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being

competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome and the vector does not include a transposon.

202. (Newly added) A vector for transformation of crop plants, which comprises a first flanking sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed.

203. (Newly added) A vector of claim 202, wherein the first and second flanking sequences are derived from tobacco.

204. (Newly added) A vector of claim 202, wherein the first and second flanking sequences are derived from *Solanum nigrum*.

205. (Newly added) A vector of claim 202, wherein the crop plant is selected from a group consisting of cotton, maize, rice, barley, lettuce, and soybeans.

206. (Newly added) A vector for transformation of crop plants, which comprises a first flanking sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed, wherein homology between the flanking sequence is between 60% and 100%.

207. (Newly added) A vector of claim 206, wherein the first and second flanking sequences are derived from tobacco.

208. (Newly added) A vector of claim 206, wherein the first and second flanking sequences are derived from *Solanum nigrum*.

209. (Newly added) A vector of claim 206, wherein the crop plant is selected from a group consisting of cotton, maize, rice, barley, lettuce, and soybeans.

210. (Newly added) A stably transformed crop plant and progeny thereof, wherein a plastid of said crop plant has been transformed by a vector which comprises a first flanking

sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed.

211. (Newly added) A stably transformed plant of claim 210, wherein homology between the flanking sequence is between 60% and 100%.

212. (Newly added) A process for stably transforming a crop plant species, comprising the steps of:

providing a crop plant species;

introducing a vector into the chloroplast genome of said crop plant species by bombardment; and allowing said crop plant species, now transformed, to grow;

wherein said vector comprises a first flanking sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed.

213. (Newly added) A process for stably transforming a crop plant species, comprising the steps of:

providing a crop plant species;

introducing a vector into the chloroplast genome of said crop plant species by bombardment; and

allowing said crop plant species, now transformed, to grow;

wherein said vector comprises a first flanking sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed, wherein homology between the flanking sequence is between 60% and 100%.

REMARKS

Interview Summary

Applicant's Attorneys thank the Examiner for the courtesy extended to Attorneys Donatiello and Yeung during the telephonic Interview on September 6, 2001. During the Interview, the parties discussed the rejections based on §112, second paragraph. Applicant's Attorneys agreed to amend the claims to reflect the changes suggested by the Examiner. The Examiner indicated that claims 198 and 199 are free of prior art. The parties also discussed the rejections based on §112, first paragraph. Applicant's Attorneys withdrew claim 2 from consideration. No agreement was reached. The Examiner indicated that evidence of other